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(54) Title: MEDICAL DEVICE COATED WITH ANTIMICROBIAL PEPTIDES

(57) Abstract: Described is a medical device for application onto or into a body of a patient, coated with one or more naturally occurring peptides or proteins or synthetic peptides and analoga thereof having antimicrobial activity. The antimicrobial peptides and proteins are preferably chosen from the group, consisting of cystatin-derived peptides, histatin-derived peptides, lactoferrin-derived peptides and specific proteinase inhibitors.

Title: Medical device coated with antimicrobial peptides.

The invention relates to a medical device for the application onto or into the body of a patient, the device being coated with one or more naturally occurring peptides or proteins or synthetic peptides and analoga thereof having antimicrobial activity.

"Medical devices for application onto or into a body of a patient" comprise devices such as endotracheal tubes, intravenous catheters, urinary catheters, draining canules, syringe needles, contact lenses, prothetic implants, such as heart valves, bone implants, voice protheses, pace makers, joint substitutes, dental implants and spinal implants. The devices can be composed of a large number of materials, known in the art, such as plastics, metals, apatites, cements etc. or of combinations of one or more of such materials. The medical devices can be coated according to many methods, known in the art, such as by direct absorption, covalent binding with the material or to a spacer, the spacer being successively absorbed or otherwise linked to the surface of the device. The coating can also be applied in the form of e.g. a gel, such as a muco-adhesive polymer or in the form of a lacquer.

A peptide or protein has "antimicrobial activity" according to the definition of the invention, when the said peptide/protein has a MIC-value of less than 10 μM. The MIC-value of a peptide/protein can be determined by incubating bacteria of the *E.coli* strain D31 in 0.25 x 100 TSB (Trypticase Soya Broth), overnight at 37 °C, with increasing concentrations of the peptide/protein in e.g. microtiter plates.

The term "protein" comprises single chained polypeptide molecules, as well as multiple polypeptide complexes, wherein the separate polypeptides are linked together in a covalent or non-covalent manner. The term "peptide" comprises peptides having a length of two or more amino acids, in particular more than 5 amino acids, preferably more than 10 amino acids.

Analoga and derivatives of proteins and peptides are herein defined as amino acid sequences, being at least 80% or more over a length of 10 amino acids, preferably 90% or more homologous to the

amino acid sequences, as described herein, as long as said analoga have the above-mentioned antimicrobial activity. The skilled person will be capable to ascertain such a homology; for this, in the art sufficient tools are known, such as the computer program GCG-BESTFIT (University of Wisconsin, US, Devereux et al, 1984, Nucl. Acids. Res. 12:387). For determining the homology, preferably the default settings of the respective computer program are used.

In the medical field there is great need for efficient antimicrobial treatment of the patient when a medical device as described above is applied onto or into the body, as the chance of obtaining an infection as a result of such application is substantial, in particular when for the application surgery is needed. When the patient obtains such an infection, very often a so-called "hospital infection" is involved, caused by a bacterial strain being resistant against the most common antibiotics and is for this reason hardly treatable. In this respect, especially endocarditis in hart valve transplants and eye infections with application of contact lenses are, among others, to be mentioned. Also, the formation of so-called "biofilms" on implants, such as the case with voice protheses and urinary catheters, are difficult to treat. A "biofilm" is a cumulation of microorganisms that are embedded in a polysaccharide matrix, adhering on solid biological and non-biological surfaces. More than 80% of the inflammations in the body are caused by biofilms. Biofilm infections are highly resistant (up to 1000 times better) against usual antimicrobial agents, and it has been shown that the immune response against such infections is initiated difficultly (Wallace et al, Rev. Infect. Dis. 5(4):657-679, 1983).

The above-mentioned problem is solved by providing a medical device according to the invention, as is worded in the main claim and the claims dependent thereon. By coating the device with an antimicrobial peptide or protein, preferably of human origin, the chance of an infection of the above-mentioned kind, including biofilm infection is effectively counteracted; further, the body of the patient appears in most cases to be not oversensitive against such peptides/proteins, in particular when these are of human origin. Further it appeared that hardly any resistant bacterial strains are formed against such peptides. It is of course clear, that the devices according to the invention are also suitable for veterinary uses; in

that case, the antimicrobial peptides are preferably originated or derived from the same species as the species to which the animal subject belongs.

Preferably, the medical device according to the invention is coated with one or more antimicrobial peptides and/or proteins, chosen from the group:

cystatin-derived peptides
histatin-derived peptides
lactoferrin-derived peptides, and
specific proteinase inhibitors.

Cystatin derived peptides

Cystatins are natural proteins, present in both human, animal and plants, being specifically capable to inhibit the class of cysteine proteinases. Said cystatins can be obtained in large amounts from chicken egg protein and from rice (oryza- cystatin). Both kinds of cystatins effectively inhibit human cysteine proteinases. These proteolytic enzymes are, among others, excreted by a number of pathogenic micro-organisms, including *Porphyromonas gingivalis*. Cysteine proteinases are also released from lysosomes from inflammatory cells, the so-called cathepsins B, H, K, L, N and S. As the acute phase proteins, cystatins are capable to inhibit the inflammatory processes. Also, they are capable to inhibit both the proteolytic activity and the growth of for example the parapathogen *Porphyromonas gingivalis*.

Cystatin peptides and analoga are synthetically produced and have both antimicrobial as inflammation inhibiting activity, making these highly suitable for coating of medical devices according to the invention in order to avoid the first inflammatory processes and infections, resulting in an enhanced acceptation of implants. It appeared that using a prothesis, coated with a peptide corresponding with the 14 amino-terminal amino acids of cystatin (SEQ ID No. 12), an infection could effectively be avoided.

Histatin derived peptides

Histatins are basic peptides (7-38 amino acids) that are particularly present in human saliva. In particular, they have an anti-fungal activity, but are inactivated in a quick manner by the bacterial proteinases in the saliva. For this reason, novel analoga (peptides) are synthesised that are not quickly inactivated and have

an increased anti-fungal activity. In addition, said peptides, being covalently coupled to a carrier appear to keep their antimicrobial activity. In addition to the fact that the said new basic peptides have an anti-fungal activity, the said newly synthesised peptides also kill bacteria having a broad spectrum, as well as viruses.

Particularly suitable for coating a medical device according to the invention is an antimicrobial peptide, having a length of 10 to 25 amino acids, and comprising a domain of at least 10 amino acids, the domain consisting of two sterically oppositely arranged opposed subdomains, the majority of the amino acids of the first subdomain being positively charged at physiological pH, and the majority of the amino acids of the second subdomain being uncharged at physiological pH. Such a peptide has an amphipathic character; it is believed that this amphipathic character is responsible for the anti-microbial activity, for reason that the peptide is thereby capable of penetrating into the bacterial membrane and causing leakages therein, resulting in killing of the bacteria.

This amphipathic character can be obtained by different confirmations in the peptide, that are elucidated below.

The structure of these peptides has a number of variations. Firstly, the domain can form an α-helix, of which at least a majority of the positions 1, 2, 5, 6, 9 (12, 13, 16, 19, 20, 23 and 24) contains a positively charged amino acid, position 8 is a positive or an uncharged amino acid and at least a majority of the positions 3, 4, 7, 10, (11, 14, 15, 17, 18, 21, 22, 25) contains an uncharged amino acid. These peptides have a lateral amphipathicity, i.e. a maximum hydrophobic moment at 100°. Stated simply, these peptides are hydrophobic on the left side and hydrophilic on the right side or vice versa. These peptides are referred to herein as "type I".

The domain can further form an α-helix, of which at least a majority of the positions 1, 2, 5, 6, 9, (12, 13, 16, 19, 20, 23 and 24) contains an uncharged amino acid, position 8 is a positive or an uncharged amino acid and at least a majority of the positions 3, 4, 7, 10, (11, 14, 15, 17, 18, 21, 22, 25) contains a positively charged amino acid. These peptides have a lateral amphipathicity i.e. a maximum hydrophobic moment at 100°. Stated simply, these peptides are hydrophobic on the right side and hydrophilic on the left side or

vice versa. These peptides are designated "type II" herein and are in principle mirror-symmetrical to type I peptides.

In addition, the domain can form an α-helix, wherein at least a majority of the positions 1 to 6 (or 7 or 8 or 9 or 10 or 11 or 12) contains an uncharged amino acid and a positively charged amino acid is found at position 7 (or 8 or 9 or 10 or 11 or 12 or 13) to 25. These peptides have a longitudinal amphipathicity, i.e. a minimum hydrophobic moment at 100°. These peptides are hydrophobic on their "top" and hydrophilic on their "bottom". Such peptides are designated "type III".

Conversely, the domain can form an α-helix, wherein at least a majority of the positions 1 to 6 (or 7 or 8 or 9 or 10 or 11 or 12) contains a positively charged amino acid and an uncharged amino acid is found at position 7 (or 8 or 9 or 10 or 11 or 12 or 13) to 25. These peptides likewise have a longitudinal amphipathicity and therefore a minimum hydrophobic moment at 100°. These peptides are hydrophobic on their "bottom" and hydrophilic on their "top". Such peptides are designated "type IV".

Finally, the domain can form a so-called β-strand and contains a positively charged amino acid on at least a majority of the positions 1, 3, 5, 7, 9 (11, 13, 15, 17, 19, 21, 23 and 25) and an uncharged amino acid on at least a majority of the positions 2, 4, 6, 8, 10, (12, 14, 16, 18, 20, 22, 24). Such a β-strand is laterally amphipathic and has a maximum hydrophobic moment at 180°. The β-strand structure is flatter than the α-helix and, stated simply, is hydrophobic on the left and hydrophilic on the right or vice versa. These are "type V" peptides.

In the β-strand confirmation, the amphipathicity can also be directed laterally or longitudinally. In a preferred embodiment of the invention, the peptides have such an amino acid composition, that the amphipathicity is oriented longitudinally, as has been shown that by this an even increased antimicrobial activity is obtained. Therefore, the two subdomains of the peptide are preferably each formed by an uninterrupted amino acid sequence, the domains proferably abutting one another.

A very good anti-microbial activity, with an activity spectrum that is very suitable for coating of medical devices according to the invention are peptides, described in SEQ ID Nos. 2-11. peptides of

SEQ ID Nos. 4 and in particular No. 5 being particularly preferred. The peptides of SEQ ID Nos. 2-11 are synthetic derivatives of the carboxy terminal of the natural histatin 5, also called DH5. The peptide having SEQ ID No. 1 corresponds with the natural amino acid sequence of the carboxy terminal of DH5 and has a satisfactory anti-microbial activity as well.

The positively charged amino acids are preferably chosen from the group consisting of ornithine (O), lysine (K), arginine (R) and histidine (H), while the uncharged amino acids are preferably chosen from the group consisting of the aliphatic amino acids glycine (G), alanine (A), valine (V), leucine (L), isoleucine (I), the amino acids with a dipolar side chain methionine (M), asparagine (N), glutamine (Q), serine (S), threonine (T), the amino acids with an aromatic side chain phenylalanine (F), tyrosine (Y), tryptophan (W). Amino acids on the border between hydrophilic and hydrophobic can be chosen from both groups or from the remaining amino acids.

Hardly any difference in activity can in principle be detected when one of the positive amino acids and/or one of the uncharged amino acids is replaced by a random amino acid. The majority of the positively charged amino acids is therefore preferably the total number of positively charged amino acids minus 1 and the majority of the uncharged amino acids is preferably the total number of uncharged amino acids minus 1.

The domain can be a part of a larger peptide but can itself also make up the entire peptide. When the domain forms part of a larger peptide, the C-terminal and/or N/terminal amino acids which are then additionally present can be random amino acids.

The peptides that can be used to coat implants according to the invention can contain further modifications. These modifications are for instance an N-terminal amide ring, for instance with acetic acid anhydride, or an alternative cleavage of the synthesis resin by which the C-terminus is modified. For this latter a replacement of the C-terminal carboxylic acid group by an amide, ester, ketone, aldehyde or alcohol group can be envisaged. Peptides with such a modification are for instance:

KRLFKELKFSLRKY-amide (peptide 12)

KRLFKELLFSLRKY-amide (peptide 13)

In addition to single peptides, oligomers can also be made. These are preferably linear oligomers of the peptides according to the invention. The coupling can be head-to-head and tail-to-tail as well as head-to-tail, either by direct synthesis or by post-synthetic enzymatic coupling. For a trans-membrane pore formation a minimum peptide length is required. Oligomers of the peptides according to the invention are double length and thereby able in principle to span the whole phospholipid double layer of the bacterial cell membrane at once. The activity of the peptide could hereby improve even further. In addition, extension of the peptides provides stabilisation of the helix conformation. A spacer must usually be inserted. In direct synthesis of head-to-tail coupled oligomers a spacer can be inserted to size by the use of a chain of unnatural amino acids of the correct length, for instance β-alanine, γ-amino butyric acid, ε-amino caproic acid, etc. Heterodifunctional coupling reagents, such as are commercially available for coupling peptide antigens to carrier proteins (for instance 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC), m-maleimidobenzoyl)-N-hydroxysuccinimide ester (MBS), N-succinimidyl 3-[pyridyldithio]propionate (SPDD) etc.) are used to make linear oligomers with an inserted spacer. For head-to-head and tail-to-tail couplings can be used trivalent amino acids such as asparagine acid (D), glutamine acid (E), ornithine (O), lysine (K), serine (S), cysteine. Such oligomers are for instance:

KRKFHEKHHSHRGYC-CYGRHSHHKEHFKRK (peptide 14)

YGRHSHHKEHFKRKC-CKRKFHEKHHSHRGY (peptide 15)

$^{\alpha}$N,$^{\varepsilon}$N-(KRKFHEKHHSHRGY)$_2$K-amide (peptide 16)

$^{\alpha}$N,$^{\varepsilon}$N-(KRLFKELKFSLRKY)$_2$K-amide (peptide 17)

$^{\alpha}$N,$^{\varepsilon}$N-(KRLFKKLKFSLRKY)$_2$K-amide (peptide 18)

Peptides 14 and 15 are obtained by synthesis of peptide 2 with an additional C-terminal respectively N-terminal cysteine, whereafter the oligomer is obtained by air oxidation. Peptides 16, 17 and 18 are obtained by making use of the Multiple Antigenic Peptide (MAP) strategy, wherein a lysine having on both the α- and on the ε-amino group an Fmoc protection was used as first amino acid on the synthesis resin, whereby two identical amino acid chains (peptides 2, 3 and 9) were synthesised simultaneously on one lysine molecule.

As alternative for the peptides, also conjugates can be used. These comprise a peptide as described above, coupled to a muco

adhesive (co)polymer. The presence of the muco adhesive (co)polymer leads to a longer residence period in mouth. The muco adhesive (co)polymer is preferably chosen from xanthane gum, polyacrylic acid, cellulose derivatives, carragenes and derivatives thereof, chitin and derivatives thereof, carragenes and derivatives thereof, chitin and derivatives thereof such as chitosan, scleroglucan and gums, such as guargum, locust beast gum etc.

It can be concluded that histatin analoga can be used as coating of implants in order to avoid infections, but only bacteria, but also of fungi and viruses.

Lactoferrin-derived peptides

Lactoferrin is one of the major peptides with antimicrobial activity in mother milk. Lactoferrin is also present in human saliva. Hydrolysed lactoferrin peptides and synthetic analoga have a potent antibacterial activity and anti fungi activity.

Lactoferrin-derived peptides can specifically be used as coating of implants in order to counteract e.g. infection of *Actinobacillus actinomycetemcomitans* and *Prevotella intermedia* (dangerous parapathogens).

Especially preferred is a peptide having an amino acid sequence, corresponding with amino acids 11-30 of the natural lactoferrin (SEQ ID No. 13) having good antimicrobial activity against an important number of bacteria and fungi that may cause hospital infections. Even more preferred is a peptide having an amino acid sequence, corresponding with the first eleven amino terminal amino acids of the natural lactoferrin (SEQ ID No. 14) showing an even improved antimicrobial activity against most bacteria and fungi that cause hospital infections, rendering the said peptide extremely well suitable as coating for medical implants according to the invention.

Specific proteinase inhibitors

In addition to inhibitors of cysteine proteinases, other specific proteinase inhibitors can be used in a coating on implants as well. The choice of proteinase inhibitors is determined by the type of implant and by the environment wherein the implant is applied. Drains and catheters that are contacted with serum or plasma can be coated with serine proteinase inhibitors (serpins) (for example the acute phase protein α1-protease inhibitors (PI) and α1-

antichymotrypsin (ACT) or with acute phase proteins, such as for example α1-antitrypsin, α2-macroglobulin and α1-acid glycoprotein.

In addition, implants that are used in supporting or connective tissue can be coated with inhibitors of metalloproteinase, including collagenase and gelatinase, that are called Tissue Inhibitor Metallo Proteinases (TIMPs). From all these enzyme inhibitors, synthetic active peptides can be produced in order to avoid bone degradation, inflammation and infection around implants.

Thus, peptides are developed having antimicrobial activity and being derived from natural occurring peptides or proteins. These peptides can have a basic or acid character and have specific activity or a broad-spectrum activity against bacteria, fungi (and yeasts) and viruses. Said peptides can be applied onto implants or drains (composed of metals, apatites, cements or plastics), the antimicrobial activity thereof being maintained. The peptide-coated implants thus have an anti infection, anti inflammation and anti repulsion activity, resulting in avoiding or inhibiting tissue loss after implantation. Further the peptide-coated implants prevent bone degradation around the implants.

Below, the invention will be further illustrated by the examples, that are however intended to illustrate the invention and in no way to limit the invention.

EXAMPLES

Peptides

All peptides tested (see tables 1 and 2) were chemically synthesised and purified according to methods known in the art.

Coating of implants, implantation in experimental animals and explantation

The method as is described in rabbits (Darouiche et al, J. Heart Valve Dis. 1998: 7:639-646) was followed using minor modifications. Hart valve implants and voice protheses were coated with a muco adhesive polymer wherein a concentration of 10 μM of the respective peptide was incorporated, and implanted in rabbits. After implantation, a bacterial or yeast inoculum (see tables 1 and 2) of 10^5 c.f.u. of a bacterial or yeast strain that was to be examined was

injected on each implant. After one week, the rabbits were sacrificed and the implants were removed.

Culturing the bacterial and yeast isolates

Isolates of the microbial and yeast strains from the explanted implants were made by culturing under suitable conditions in brain-heart-infusion broth and plating on an agar nutrient plate, suitable for the respective bacterial or yeast strain. After incubation during 24-48 h the number of individual colonies was determined, being shown in tables 1 and 2.

Table 1 Microbial growth on voice protheses, coated with an antimicrobial peptide

Micro-organism	Peptide						
	DH5 SEQ ID	DHVAR1 SEQ ID	DHVAR4 SEQ ID	DHVAR5 SEQ ID	S1-15 SEQ ID	L17-30 SEQ ID	L1-11 SEQ ID
	1	2	4	5	12	13	14
Candida tropicalis	+	-	-	-	+/-	+/-	-
Candida albicans	+	-	-	-	+/-	+/-	-
Candida krusei	+	+/-	-	-	+/-	+/-	-
Candida humicola	+	-	-	-	+/-	+/-	-
Streptococcus anginosus	+	-	-	-	+/-	-	-
Streptococcus salivarius	+	-	-	-	+/-	-	-
Rothia dentocariosa	+	+/-	-	-	+/-	+/-	-
Staphylococcus aureus	+	+/-	-	-	+/-	+/-	-
Staphylococcus epidermis	+	-	-	-	+/-	-	-
Stomatococcus mucaliginosus	+	-	-	-	+/-	-	-
Escherichia coli	+	+/-	-	+/-	+/-	-	-
Enterococcus faecium	+	-	-	-	+/-	-	-

Table 2 Microbial growth on heart valve implants, coated with an antimicrobial peptide

Micro-organism	Peptide						
	DH5 SEQ ID 1	DHVAR1 SEQ ID 2	DHVAR4 SEQ ID 4	DHVAR5 SEQ ID 5	S1-15 SEQ ID 12	L17-30 SEQ ID 13	L1-11 SEQ ID 14
Staphylococcus aureus	+	+/	-	-	+/-	+/-	-
Escherla coli	+	+/-	-	-	+/-	-	-
Steptococcus viridans	+	+/-	-	-	+/-	+/-	-
Enterococcus sp.	+	+/-	-	-	+/-	-	-

\+ : more than 10 individual colonies per culture plate (diameter 8 cm)
+/- : less than 10 individual colonies per culture plate (diameter 8 cm)
\- : no colonies found

The above tables show that in particular DHVAR4, DHVAR5 and L1-11 show excellent antimicrobial activity as coating on an implantate. DHVAR1, S1-15 and L17-30 are also suitable for a more limited application. Similar results were obtained with DHVAR2, 3, and 6-11. DH5 provides less antimicrobial protection than the other peptides; on average 15-20 colonies were found per plate. Uncoated control-implants usually show significantly more colonies, up to above 100 colonies per plate.

CLAIMS

1. Medical device for application onto or into a body of a patient, the device being coated with one or more naturally occurring peptides or proteins or synthetic peptides and analoga thereof having antimicrobial activity.

2. Medical device according to claim 1, the antimicrobial peptides and proteins being chosen from the group, consisting of:
cystatin-derived peptides,
histatin-derived peptides,
lactoferrin-derived peptides, and
specific proteinase inhibitors.

3. Medical device according to claim 2, wherein the antimicrobial peptide has a length of 10-25 amino acids, comprising a domain of at least 10 amino acids, the domain consisting of two sterically oppositely arranged subdomains, the majority of the amino acids of the first subdomain being positively charged at physiological pH and the majority of the amino acids of the second subdomain being uncharged at physiological pH.

4. Medical device according to claim 3, wherein the two subdomains of the peptide are each formed by an uninterrupted amino acid sequence.

5. Medical device according to any of the preceding claims, wherein the peptide is chosen from the group:

KRKFHEKHHSHRGY	(SEQ ID No. 1)	DH5
KRLFKELKFSLRKY	(SEQ ID No. 2)	DHVAR-1
KRLFKKLKFSLRKY	(SEQ ID No. 3)	DHVAR-3
KRLFKKLLFSLRKY	(SEQ ID No. 4)	DHVAR-4
LLLFLLKKRKKRKY	(SEQ ID No. 5)	DHVAR-5
KRLFKELLFSLRKY	(SEQ ID No. 6)	DHVAR-6
KRLFKELKKSLRKY	(SEQ ID No. 7)	DHVAR-7
KRLFKELLKSLRKY	(SEQ ID No. 8)	DHVAR-8
OOLFOELOOSLOOY	(SEQ ID No. 9)	DHVAR-9
OOLFOELLOSLOOY	(SEQ ID No. 10)	DHVAR-10
KRLFKKLKFSLRKY	(SEQ ID No. 11)	DHVAR-11
SSSKEENRIIPGGI	(SEQ ID No. 12)	S1-15
FKCRRWQWRMKKLG	(SEQ ID No. 13)	L17-30
GRRRRSVQWCA	(SEQ ID No. 14)	L1-11

6. Use of one or more naturally occurring peptides or proteins or synthetic peptides and analoga thereof having antimicrobial activity for coating of medical devices for application onto or into a body of a patient, in particular implants and contact lenses.

7. Use according to claim 6, wherein the antimicrobial peptides and proteins are chosen from:
cystitin-derived peptides,
histatin-derived peptides,
lactoferrin-derived peptides, and
specific proteinase inhibitors.

8. Use according to claim 7, wherein the antimicrobial peptide has a length of 10-25 amino acids, comprising a domain of at least 10-25 amino acids, comprising a domain of at least 10 amino acids, the domain consisting of two sterically oppositely arranged subdomains, wherein the majority of the amino acids of the first subdomain being positively charged at physiological pH and the majority of the amino acids of the second subdomain being uncharged at physiological pH.

9. Use according to claim 8, wherein the two subdomains of the peptide are each formed by an uninterrupted amino acid sequence.

10. Use according to any of claims 6-9, wherein the peptide is chosen from the group consisting of:

KRKFHEKHHSHRGY (SEQ ID No. 1) DH5
KRLFKELKFSLRKY (SEQ ID No. 2) DHVAR-1
KRLFKKLKFSLRKY (SEQ ID No. 3) DHVAR-3
KRLFKKLLFSLRKY (SEQ ID No. 4) DHVAR-4
LLLFLLKKRKKRKY (SEQ ID No. 5) DHVAR-5
KRLFKELLFSLRKY (SEQ ID No. 6) DHVAR-6
KRLFKELKKSLRKY (SEQ ID No. 7) DHVAR-7
KRLFKELLKSLRKY (SEQ ID No. 8) DHVAR-8
OOLFOELOOSLOOY (SEQ ID No. 9) DHVAR-9
OOLFOELLOSLOOY (SEQ ID No. 10) DHVAR-10
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FKCRRWQWRMKKLG (SEQ ID No. 13) L17-30

SEQUENCE LISTING

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<223> Description of Artificial Sequence:antimicrobial peptide

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1 5 10

<210> 9
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:antimicrobial peptide

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1 5 10

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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:antimicrobial peptide

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1 5 10

<210> 11
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<223> Description of Artificial Sequence:antimicrobial peptide

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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:antimicrobial peptide

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<223> Description of Artificial Sequence:antimicrobial peptide

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<210> 14
<211> 11
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:antimicrobial peptide

<400> 14
Gly Arg Arg Arg Arg Ser Val Gln Trp Cys Ala
1 5 10

GRRRRSVQWCA (SEQ ID No. 14) L1-11

INTERNATIONAL SEARCH REPORT

tional Application No
PCT/NL 01/00019

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/34 A61L29/08 A61L31/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L C07K A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	EP 0 990 924 A (JOHNSON & JOHNSON VISION PROD) 5 April 2000 (2000-04-05) paragraph '0006! - paragraph '0011! examples 1,2	1-10
X	WO 98 40091 A (UNIV NEW YORK) 17 September 1998 (1998-09-17) page 4, line 22 - line 30	1,6
X	US 6 008 195 A (SELSTED MICHAEL E) 28 December 1999 (1999-12-28) column 19, line 27 - line 37	1,6
X	WO 99 01089 A (UNIV BROWN RES FOUND; VALENTINI ROBERT F (US)) 14 January 1999 (1999-01-14) claims 1,2	1,6

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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'A' document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
21 June 2001	29/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Heck, G

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INTERNATIONAL SEARCH REPORT

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